Validation of Microbial Source Tracking Methods

Valerie J. Harwood, Don Stoeckel & Shiao Wang

USF UNIVERSITY OF SOUTH FLORIDA

USGS science for a changing world

THE UNIVERSITY OF SOUTHERN MISSISSIPPI 1910

Expectations of MST: Stage 1

- **Wild optimism**

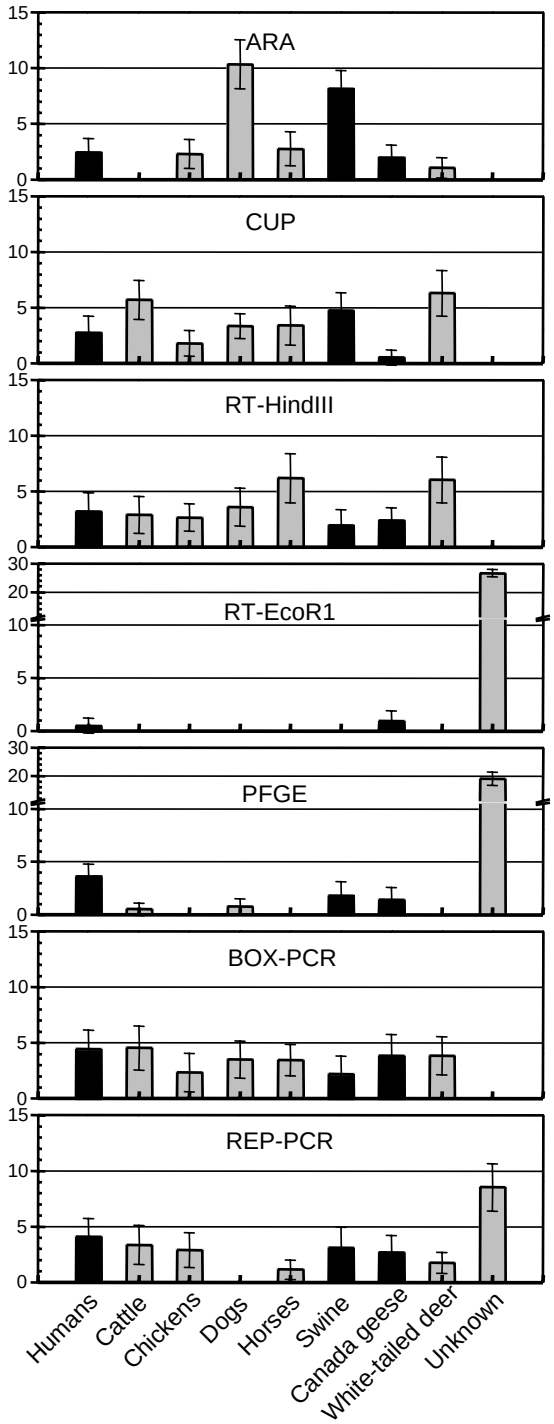
Expectations of MST: Stage 2

Uh-oh…not so fast!

SCCWRP study 2003; Stoeckel et al 2004 *E. coli* libraries

30 *E. coli* isolates were chosen randomly from the challenge sample set

- 10 human
- 10 swine
- 10 Canada goose



Expectations of MST Stage 3

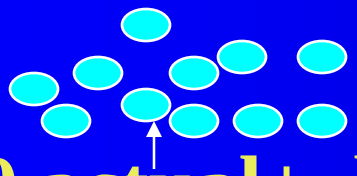
"Optimistic skepticism" **Stoeckel 2006**

- **Assess sensitivity and specificity**
- **Validation of library-dependent methods must include isolates from independent reference materials (e.g. fecal samples)**
- **Validation of library-independent methods must include composites containing fecal material from target or composites from nontarget sources**

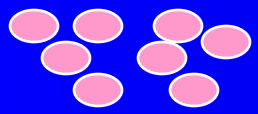
Sensitivity

Ability to detect target when present

% of actual + that are detected



10 actual+, but only 8 detected



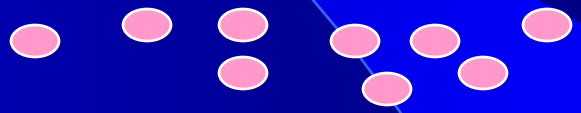
8/10 = 80%

False -

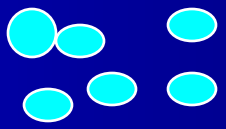
Specificity

Confidence in a positive result

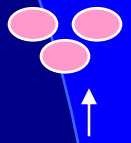
% of detected positives were actual positives



9 detected +, but only 6 actual +

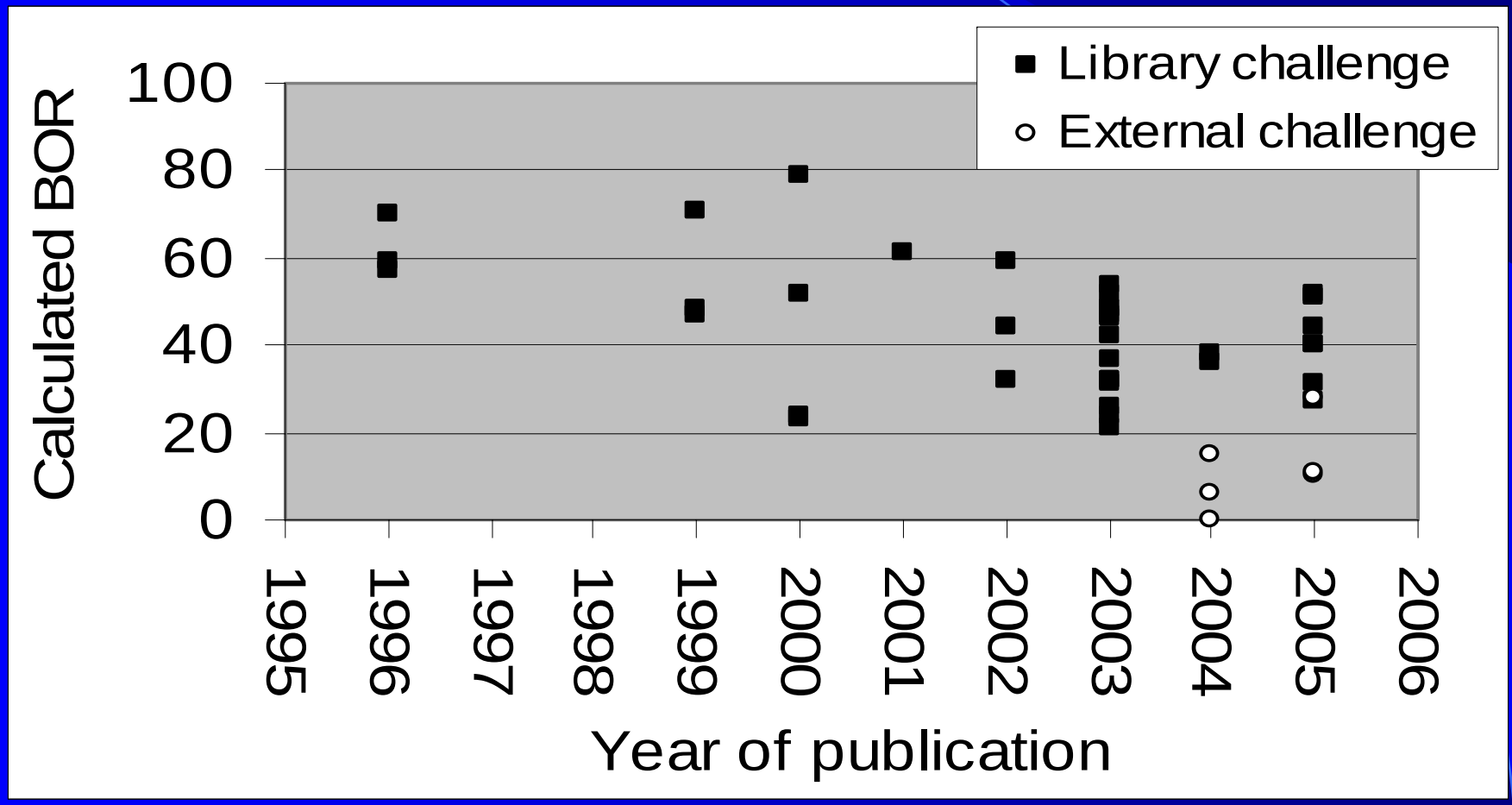


6/9 = 66.7%



False +

BOR of Library-Based Methods



Library-Independent Markers

- Lack the historical record of library-dependent methods.
- Validation results (sensitivity and specificity) continue to be compiled (we hope!).

Field Validation Needed!

- **Effects of differential survival/ rapid die-off in secondary habitat**
- **Matrix effects such as humic substances on PCR**

Confirm Successful Methodology Transfer!

External Measures of Method Success Should Be REQUIRED in Publications and for Management Reports (Defensibility)

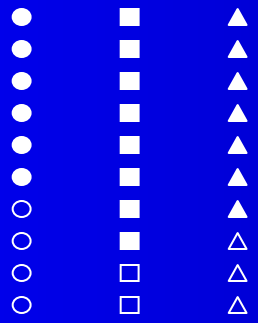
Comparing Apples to Oranges - How to Compare Method Accuracy When the Possible Number of Source Categories is Different?

Example: Study A splits all observations into two possible source categories, e.g. animal and human, and the method correctly assesses fecal source in 74% of samples.

Study B splits all observations into four possible source categories, and the fecal source is assessed correctly in 59% of samples.

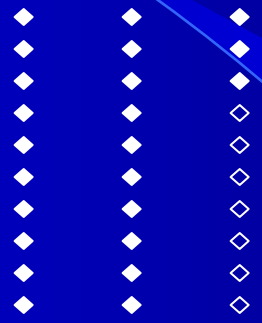
"Benefit Over Random"

Nonhuman source isolates



Correct: 210 of 300
RCC: 70%

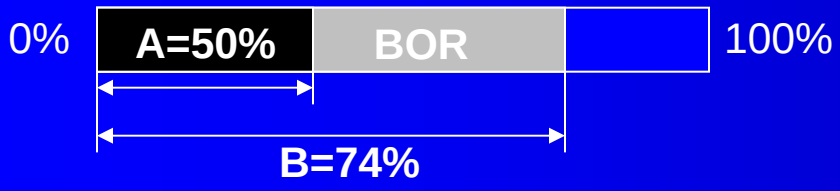
Human-source isolates



Correct: 230 of 300
RCC: 77%

ARCC: 74%
Categories: Two
Random: 50%
BOR: 74%-50%=24%

Classification accuracy



A=measure of random classification (e.g. 1/*k*)
B=measure of accuracy (e.g. ARCC)

Benefit over random (BOR) = B - A




Correct:	50 of 100	80 of 100	30 of 100	60 of 100
RCC:	50%	80%	30%	60%

Each symbol represents ten isolates. Dark symbols were correctly classified Open symbols were incorrectly classified

ARCC: 55%
Categories: Four
Random: 25%
BOR: 55%-25%=30%

By comparison, the two-way split had ARCC 74%, BOR 24%